## REMARKS

The Office Action dated March 17, 2004, has been carefully reviewed. Claims 1-30 are pending in this application. Reconsideration of this application is respectfully requested in light of the following discussion.

**Bibliographic Information Pertaining to Reference 33**

On page 2, lines 1-7, the Examiner requests that the Applicants provide an additional PTO-1449 form that sets forth the bibliographic information of reference 33 (i.e., Bacterial Quinoproteins Glucose Dehydrogenase and Alcohol Dehydrogenase). Applicants acknowledge the Examiner's request and have provided another PTO-1449 form which only lists this reference along with its bibliographic information.

**Certified copy of European Application 00127294.7**

On page 2, lines 8-13, the Examiner alleges that a certified copy of European Application 00127294.7 has not been filed. In response, the Applicants attach herewith a certified copy of European Application 00127294.7.

**Objection to Figures 1, 2, and 4**

On page 2, lines 14-19, the Examiner alleges that Figures 1, 2, and 4 do not comply with 37 CFR 1.821-1.825, and suggests that SEQ ID NO(s): be added to the description on page 6. In response to the Examiner's suggestion, as indicated above, Applicants have added SEQ ID NO(s): to the description on page 6.

**Objection to Claims 28-30 under 37 CFR § 1.75(c)**

On page 2, line 20, through page 3, line 3, the Examiner has objected to claims 28-30 as allegedly being in improper form "because a multiple dependent claim should depend only one other claim in the alternative and a multiple dependent claim should not depend to another multiple dependent claim." As indicated above, claims 28 -30 have been amended to address the Examiner's concern. Claims 28-30 are now believed to be in condition for allowance and such action is respectfully requested.

**Discussion of Claims 1, 6, 8, 9, 13, 16, 18, 20 and 27 with Respect to 35 U.S.C. § 112, 2nd Paragraph**

On page 3, lines 4-23, the Examiner has rejected claims 1, 6, 8, 9, 13, 16, 18, 20, and 27 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. The Examiner specifically states:

> Claims 1 and 6 are confusing and apparently incorrect in the recitation of "EC 1.1.99.17". This enzyme has been transferred to 1.1.5.2, as noted in the enclosed NiceZyme entry from ExPASy.

As suggested by the Examiner, claims 1 and 6 have been amended to delete "EC 1.1.99.17" and recite "EC 1.1.5.2." In addition, as indicated above, Applicants have amended the specification of the subject application to indicate that the designation of EC 1.1.99.17 has been changed to EC 1.1.5.2.

On page 3, lines 11-15, the Examiner has rejected claims 1, 6, 8, 9, 13, 16, 18, and 20 as allegedly being indefinite in the recitation of "PQQ" and has stated that the recitation of "pyrroloquinoline quinone (PQQ)-dependent..." in claim 1 would overcome this rejection. In response, claim 1 has been amended in line with the Examiner's suggestion.

On page 3, lines 16-23, the Examiner further alleges that claim 8 is incorrect. In particular, the Examiner states:

> Claim 8 is incorrect in the recitation of "A. baumanni", which should be "*A. baumanni*". The claim is also indefinite and confusing in the recitation of "is isolated from a strain of the Acinetobacter species group consisting of *A. calcoaceticus* and A. baumanni". The instant recitation is drawn to "a strain" and then lists two strains. A recitation of "is isolated from a strain of Acinetobacter selected from the group consisting of *A. calcoaceticus* and A. baumanni", or some similar recitation would overcome this rejection.

Claim 8 has been amended herein to overcome the Examiner's rejection.

On page 3, lines 24, through page 4, line 2, the Examiner has rejected claim 9 and 13 as allegedly being indefinite and not in conformity with the sequence rules (37 CFR §1.821 -1.825). In particular, the Examiner states:

> ....the sequence of the mature "*A. calcoaceticus*" does not have its SEQ ID NO: listed. For purposes of this examination it is presumed that it is SEQ ID NO: 24.

Applicants have amended claims 9 and 13 to recite SEQ ID NO: 24.

On page 4, lines 3-7, the Examiner has further rejected claim 9 as allegedly being indefinite and confusing in the recitation of the group comprising positions 348 and 428. Claim 9 has been amended to recite "at least one amino acid residue substitution at an amino acid position selected from a group consisting of positions 348 and 428."

On page 4, lines 8-12, the Examiner has pointed out a typographical error in claim 13 and has also rejected claim 13 as allegedly being indefinite in the recitation of "T348 or N428 is replaced." Claim 13 has been amended to correct the typographical error and to more clearly state "that at least one of the amino acid residues, T348 or N428, is replaced with another amino acid."

On page 4, lines 13-17, the Examiner has also rejected claims 16, 18, and 20 as allegedly being confusing and inconsistent in the recitation of "WPXaaVAPS", "TAGXaaVQK" and "ADGXaaNGL", respectively. The abbreviations in claims 16, 18, and 20 have been amended herein to be consistent with the three-letter sequence listing.

On page 4, lines 18-23, the Examiner has rejected claim 27 as allegedly being indefinite in the recitation of "variants with the construction of claim 26..." and in the absence of antecedent basis for "construct of claim 26". The Examiner notes that a recitation of "variants comprising culturing a host cell containing the expression vector of claim 26" or some similar recitation would overcome this rejection. Claim 27 has been amended to read as follows:

> 27. A process for producing s-GDH variants comprising expressing the expression vector of claim 26 in a cell-free peptide synthesis system under conditions suitable for production of the said enzyme variants.

In light of the amendment to claim 27, Applicants respectfully request that the Examiner withdraw the instant rejection.

With the foregoing amendments, it is believed that the claims are now in condition for allowance and such action is respectfully requested.

**Discussion of Claims 1-27 with Respect to 35 U.S.C. § 112, 1st Paragraph**

On page 5, line 3, through page 6, line 2, the Examiner has rejected claims 1-27 under 35 U.S.C. § 112, first paragraph. More particularly, on page 5, beginning with the first paragraph, of the Office Action, the Examiner states:

> ...The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to the invention

> commensurate in scope with these claims make and/or use the claimed invention.
>
> The instant claims are not limited to a particular PQQ-dependent glucose dehydrogenase or to a particular source of the enzyme, and are not limited to particular mutations or they are too broad to cover the requirement in claim 1 that the activity be at least two-fold higher with glucose than with another sugar. The specification does not teach one of ordinary skill in the art how to make all of the enzymes and/or polynucleotides of the instant claims, only how to make the PQQ-dependent glucose dehydrogenase from *A. calcoaceticus* having particular amino acid changes. Exactly what affect a change in a particular amino acid, or the corresponding polynucleotide encoding it, will have on an enzyme's activity is not predictable with any certainty. Therefore the claims should be limited to the embodiments taught in the instant specification.
>
> It is noted that Table 1 does not show the effect of a mutation at only position 428, T348A is shown to have 7% as much activity with maltose as glucose, a T348S mutation is not shown alone and an mutation of position 428 to any amino acid alone is not shown. Likewise, all of the possible combinations of claims 11 and 13-15 are not shown. claims 16-21 read on any PQQ-dependent glucose dehydrogenase comprising the indicated short sequences given in the claims and the specification does not teach one of ordinary skill how to make all of the embodiments of these claims.

Respectfully, the Examiner's contentions regarding enablement are not believed to be pertinent to the invention as claimed.

One way to enable the present invention involves a wild-type PQQ-dependent s-GDH gene being isolated from an appropriate bacterium and cloned into an appropriate expression vector, mutations being generated in the cloned PQQ-dependent s-GDH gene, active clones expressing a mutated PQQ-dependent s-GDH gene in a host cell being selected by a screening process, mutated PQQ-dependent s-GDH genes being sequenced and expressed, and mutant PQQ-dependent s-GDH being purified and assayed for glucose substrate specificity. As indicated in the specification on page 15, lines 8-10, suitable expression vectors containing the desired coding and control sequences may be constructed using standard recombinant DNA techniques known in the art, many of which are described in Sambrook et al. (1989). Further, as described in the specification on page 15, lines 21-25, expression vectors may be introduced into host cells by various methods known in the art. As pointed out in the specification on page 16, lines 14-19, not all expression vectors and DNA regulatory sequences would function equally well to express the DNA sequences of the Applicants' invention; neither will all host cells function equally well with the same expression system. However, one of ordinary skill in the art may make a selection among expression vectors, DNA regulatory sequences, and host cells; conduct random and saturation mutagenesis; generate and screen s-GDH mutants; and assay purified, mutant PQQ-dependent s-GDH for glucose substrate specificity using the guidance provided by the

Applicants without undue experimentation and without departing from the scope of the claimed invention.

Although Applicants have focused on isolating mutants of PQQ-dependent s-GDH from *A. calcoaceticus*, the amino acid sequence of wild-type s-GDH from *A. baumanni* shows significant sequence homology, as is evident from Figure 2 in the specification. Further, as indicated on page 1, lines 25-27 of the specification, PQQ-dependent s-GDHs have been found only in the periplasmatic space of *Acinetobacter* strains, which tends to suggest a highly conserved s-GDH amino acid sequence among all *Acinetobacter* strains. In addition, as indicated on page 2, lines 1-8 of the specification, the deduced amino acid sequences of several as yet uncharacterized proteins from *E. coli*, *Synechocystis sp.*, *P.aeruginosa*, and *Bordetella pertussis* are closely related to *A. calcoaceticus* s-GDH, the likely results of which are similar structures and catalysis of similar PQQ-dependent reactions. Thus, Applicants' specification would enable a skilled artisan to make mutant PQQ-dependent s-GDH from a variety of bacterial strains, not just *A. calcoaceticus*.

Accordingly, the Examiner's rejection of claims 1-27 under 35 U.S.C. § 112, first paragraph, for alleged lack of enablement, is believed to be improper because, as demonstrated by Applicants in Examples 1-7, a person skilled in the art would be able to use the claimed invention. Thus, Applicants request that the Examiner withdraw the present rejection.

**Discussion of Claims 1-7 and 22-26 with Respect to 35 U.S.C. § 102(b)**

On page 6, line 28, through page 7, line 8, the Examiner has rejected claims 1-7 and 22-26 under 35 U.S.C. § 102(b) as allegedly being anticipated by either of Cleton-Jansen, et al. (U) or Sode (4). In particular, beginning on page 6, the Examiner states:

> Cleton-Jansen, et al., teach a form of PQQ-dependent glucose dehydrogenase that will only oxidize glucose (P1) and one that will also oxidize maltose (P2). The reference characterizes P2 as a mutant of P1 but since all proteins in nature are constantly undergoing mutation, P1 could be characterized as a mutant of P2. Soda teaches in Table 1 a series of mutants of PQQ-dependent glucose dehydrogenase that meet the requirements of the instant claims. All of the mutant proteins have at least two fold higher activity against glucose than maltose. It is maintained that the enzyme taught by the instant references are the same as those of the instant claims, absent very convincing proof to the contrary. The polynucleotide sequence is taught in Fig. 3 of Cleton-Jansen and the nucleotide sequence of Sode is known since it is a known variation of a known nucleotide. The patent office does not have facilities to assay enzymes and see what characteristics they have.

Applicants respectfully point out that each independent claim of the instant application currently reads as follows:

1. A mutant of the soluble form of EC 1.1.5.2 also known as pyrroloquinoline quinone (PQQ)-dependent soluble glucose dehydrogenase (s-GDH) said mutant characterized in that it has an at least two-fold increased substrate specificity for glucose, as compared to at least one other selected sugar substrate.

6. A mutant of the soluble form of EC 1.1.5.2 also known as PQQ-dependent soluble glucose dehydrogenase (s-GDH) said mutant characterized in that
the substrate specific reactivity towards glucose is essentially comparable to that of the wild-type enzyme, and
the substrate specific reactivity towards maltose is 30% or less as compared to the wild-type enzyme.

16. A mutant protein of PQQ-dependent s-GDH comprising the amino acid sequence of TrpProXaaValAlaProSer (SEQ ID NO: 1), wherein said Xaa residue is an amino acid residue other than threonine.

18. A mutant protein of PQQ-dependent s-GDH comprising the amino acid sequence of ThrAlaGlyXaaValGlnLys (SEQ ID NO: 2), wherein said Xaa residue is an amino acid residue other than asparagine.

20. A mutant protein of PQQ-dependent s-GDH comprising the amino acid sequence of AlaAspGlyXaaAsnGlyLeu (SEQ ID NO: 3), wherein said Xaa residue is an amino acid residue other than glutamine.

In light of the above, the Examiner will appreciate that each of the independent claims is directed to the soluble form of GDH. Accordingly, each dependent claim is also directed to the soluble form of GDH. Applicants further point out that Cleton-Jansen, et al. (U) and Sode (4) both teach only the membrane-bound form of GDH. The Examiner is respectfully reminded of the differences between the membrane-bound and soluble form of GDH; see, for example, page 1, lines 22-27, and page 2, lines 9-16, within the specification. Therefore, since Cleton-Jansen, et al. (U) and Sode (4) only teach the membrane-bound form of GDH, neither of these relied upon references disclose each and every claim element of any of the claims of the present application. Accordingly, these relied upon references do not anticipate any of the instant claims. Thus, Applicants request the Examiner to withdraw the present rejection.

**Discussion of Claims 1-7 and 22-27 with Respect to 35 U.S.C. § 103(a)**

On page 7, lines 9-19, the Examiner has rejected claims 1-7 and 22-27 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Cleton-Jansen, et al. (U) or Sode (4). In particular, on page 7 of the Official Action, the Examiner states as follows:

> The instant references are characterized *supra*. It is maintained that if the vector of claim 26 could not be used in a cell-free system then one of ordinary skill in the art would know how to modify it so that it would using the general knowledge in the prior art. Therefore, it would have been obvious to one of ordinary skill in the art to produce a vector that could be used in either a host cell or cell-free system in view of the instant reference and the general knowledge in the art. The motivation would have been to produce the enzyme so as to further study it or to use it in glucose detection.

As indicated above in Applicants' response to the Examiner's rejection of claims 1-7 and 22-26 under 35 U.S.C. § 102(b), membrane-bound and soluble PQQ-dependent glucose dehydrogenase represent two completely different molecules that would require different expression vectors as a means of producing enzyme for study or use in glucose detection. As a result, it would not have been obvious to one of ordinary skill in the art, based on the teachings of Cleton-Jansen, et al. (U) and Sode (4), to produce a vector that could be used in either a host cell or cell-free system to produce soluble PQQ-dependent glucose dehydrogenase enzyme so as to further study it or use it in glucose detection. Accordingly, the Examiner has not established a proper prima facie case of obviousness. Thus, Applicants request the Examiner to withdraw the present rejection.

**Discussion of Claims 1-5, and 13-27 with Respect to 35 U.S.C. § 102(b)**

On page 7, lines 20-25, the Examiner has rejected claims 1-5 and 13-27 under 35 U.S.C. § 102(b) as allegedly being anticipated by Cleton-Jansen, et al. (15). The Examiner contends that the instant reference teaches in Table 2 the mutant PP2403 of PQQ-dependent glucose dehydrogenase, which has 0.3 units/ml of activity against glucose and 0.0 units/ml activity against maltose. The Examiner further contends that all of the other requirements of the instant claims are taught by the reference.

Cleton-Jansen, et al. (15) teaches several (mutant) strains of *A. calcoaceticus*. From these strains cell-free extracts are prepared and the overall GDH activity is assessed in vitro. The cell-free extract of mutant strain PP2403 exhibits 0.3 units/ml of activity against glucose and 0.0 units/ml of activity against maltose and lactose. Mutant strain PP2403 and the other mutant strains listed in Table 2 are the result of one or more insertions, whereby a gene for kanamycin (an antibiotic) resistance has been inserted into the structural genes for

PQQ-dependent glucose dehydrogenase. The mutant strains listed in Table 2 were produced for the express purpose of determining the role of membrane-bound PQQ-dependent glucose dehydrogenase, designated GDH-A, and soluble PQQ-dependent glucose dehydrogenase, designated GDH-B, in vivo and in vitro as evidenced by monosaccharide and disaccharide oxidation. Mutant strain PP2403 harbors an insertion mutation in the gene for GDH-B (i.e., a disrupted GDH-B gene), the result of which is measurable glucose dehydrogenase activity by GDH-A, i.e., membrane-bound PQQ-dependent glucose dehydrogenase. Thus, in PP2403, Cleton-Jansen, et al. (15) teaches a mutant strain wherein glucose oxidation is attributable to membrane-bound PQQ-dependent glucose dehydrogenase activity, not to soluble PQQ-dependent glucose dehydrogenase activity or a combination thereof. As a result, it is respectfully submitted that the enzyme taught by Cleton-Jansen, et al. (15) in mutant strain PP2403, listed Table 2, is not the same as those of the instant claims, all of which are directed to a soluble PQQ-dependent glucose dehydrogenase. Thus, Applicants request the Examiner to withdraw the present rejection.

**Discussion of Claims 16, 18 and 20-21 with Respect to 35 U.S.C. § 102(b)**

On page 8, lines 1-5, the Examiner has rejected claim 16 under 35 U.S.C. § 102(b) as allegedly being anticipated by Murphy, et al.(V). The Examiner contends that the instant reference teaches a protein with SEQ ID NO:1 where Xaa is not threonine. The Examiner further contends that since the reference meets the requirements of the claim as to sequence, it meets the requirements of the entire claim since it does not matter what the protein is called.

The protein identified in Murphy, et al. (V) is a 305 amino acid-containing protein from Streptomyces coelicolor, amino acid residues 292-298 of which represent SEQ ID NO:1 where Xaa is not threonine. Murphy, et al. (V) does not describe the protein as a s-GDH nor is any information provided as to the protein's dependence on PQQ. The Examiner is respectfully directed to the preamble of claim 16, which specifically recites "A mutant protein of PQQ-dependent s-GDH...", and which Applicants regard as a claim element. Accordingly, the instant reference does not meet the requirements of the entire claim. Thus, Applicants request the Examiner to withdraw the present rejection.

On page 8, lines 6-10, the Examiner has rejected claim 18 under 35 U.S.C. § 102(b) as allegedly being anticipated by Lamelp et al. (W). Applicants respectfully point out that Kaneko, et al. (W) is believed to be the reference on which the Examiner has based the

instant rejection. The instant reference is held to teach a protein with SEQ ID NO:2 where Xaa is not asparagine. The Examiner further contends that since the reference meets the requirements of the claim(s) as to sequence, it meets the requirement of the entire claims since it does not matter what the protein is called.

The protein identified in Kaneko, et al. (W) is a 422 amino acid-containing protein from the unicellular cyanobacterium Synechocystis sp., amino acid residues 48-54 of which represent SEQ ID NO:2 where Xaa is not asparagine. Kaneko, et al. (W) does not describe the protein as a s-GDH nor is any information provided as to the protein's dependence on PQQ. The Examiner is respectfully directed to the preamble of claim 18, which specifically recites "A mutant protein of PQQ-dependent s-GDH...", and which Applicants regard as a claim element. Accordingly, the instant reference does not meet the requirements of the entire claim. Thus, Applicants request the Examiner to withdraw the present rejection.

On page 8, lines 11-15, the Examiner has rejected claims 20-21 under 35 U.S.C. § 102(b) as allegedly being anticipated by Granger, et al. (X). The Examiner contends that the instant reference teaches a protein with SEQ ID NO:3 where Xaa is not glutamine. The Examiner further contends that since the reference meets the requirements of the claim as to sequence, it meets the requirements of the entire claim since it does not matter what the protein is called.

The protein identified in Granger, et al. (X) is a 507 amino acid-containing protein from Trichomonas foetus, amino acid residues 293-299 of which represent SEQ ID NO:3 where Xaa is not glutamine. Granger, et al. (X) does not describe the protein as a s-GDH nor is any information provided as to the protein's dependence on PQQ. The Examiner is respectfully directed to the preamble of claim 20 which specifically recites "A mutant protein of PQQ-dependent s-GDH...", and which Applicants regard as a claim element. Accordingly, the instant reference does not meet the requirements of the entire claim. Thus, Applicants request the Examiner to withdraw the present rejection.

## CONCLUSION

In view of the foregoing amendments and remarks, it is submitted that this application is in condition for allowance. Action to that end is hereby solicited.

Respectfully submitted

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